REMARKS

Claims 1-22 are pending in the subject application. The Examiner has withdrawn claims 8-22 from consideration as being drawn to a non-elected species. Claims 1-7 have been examined on the merits. Claims 1, 6 and 7 have been amended to more particularly point out and clearly claim the subject matter the Applicants consider their invention.

Support for the amendments may be found throughout the application but particularly on p. 9, lines 17-25, p. 10, lines 1-9, p. 12, lines 11-24, p. 24, lines 12-22, p. 26, lines 13-16, p. 41, lines 4-17, and p. 43, line 20 to p. 44, line 19.

The Examiner has objected to various informalities in the claims and specification. Appropriate correction is made herewith.

1. Rejections under 35 U.S.C. § 112

1.1. Rejection of claims 1-7 under 35 U.S.C. § 112, Second Paragraph Indefinitness

The Examiner rejects claim 1 as vague and indefinite because it is allegedly unclear as to the use of the terms MAD2 and TUB1 as a sole means of identifying the genes recited in the claims.

Applicants respectfully disagree and assert that the claim recitations "MAD2" and "TUB1" are clear on their face and assert that the Examiner has provided no evidence or basis for the statement that different labs refer to these genes by different designations.

The recitation "TUB1", refers to a gene that encodes the ubiquitous protein alpha-tubulin. *See* p. 40, lines 35-26. A simple search of the iHOP (Information Hyperlinked Over Proteins; http://www.pdg.cnb.uam.es/UniPub/iHOP/; *see* Hoffmann, R., Valencia, A. A Gene Network for Navigating the Literature. *Nature Genetics* 36, 664 (2004)) database with the term "TUB1" yields a range of accession numbers for the TUB1 gene and protein sequences: UniProt P09733, NCBI Gene 854889, NCBI RefSeq NP_013625.1, NCBI Accession AAA35180 and CAA86653, *see* Exhibit A. Moreover, the search yields a long list of scientific publications describing the structural and functional attributes of TUB1. Finally, the search identifies and hyperlinks to similar information on TUB1 homologs in humans, mouse, *Drosophila*, *C.elegans* and *Arabidopsis*.

Additionally, one of skill in the art would recognize that "MAD2" refers to a yeast mitotic spindle checkpoint component gene. A simple search of the iHOP database with the term "MAD2" yields a range of accession numbers for the MAD2 gene and protein sequences: UniProt P40958; NCBI Gene 853422; NCBI RefSeq NP_012504.1; NCBI Accession AAA21385, CAA89321 see Exhibit B. Moreover, the search yields a long list of scientific publications describing the structural and functional attributes of MAD2. Finally, the search identifies and hyperlinks to similar information on MAD2 homologs in humans (Exhibit B, Paper No. 1) , mouse (Exhibit B, Paper No. 2), *Drosophila* (Exhibit B, Paper No. 3), *C.elegans* (Exhibit B, Paper No. 4) and *Arabidopsis* (Exhibit B, Paper No. 5).

In view of this evidence documenting that one of skill in the art would readily understand what the terms "TUB1" and MAD2" refer to Applicants respectfully request withdrawal of this rejection.

The Examiner rejects claims 1-2, and 7 as being indefinite for allegedly failing to recite a control step. Applicants respectfully assert that the amendments to claim 1 fully addresses this ground for rejection.

1.2. Rejection of claims 1-7 under 35 U.S.C. § 112, First Paragraph, Written Description

The Examiner rejects claims 1-7 under 35 U.S.C. § 112, first paragraph, for allegedly encompassing subject matter which was not adequately described in the application. The Examiner asserts that the claim recitation "mutated MAD2" including analogs and homologs thereof, generically covers a large number of species. However, the Examiner alleges that the disclosure only exemplifies one yeast MAD2 mutant and that no structural information is provided to define the genus of molecules. Applicants respectfully disagree.

With regard to "homologs," the Examiner has pointed out that the term has been defined in the specification (Office Action of 10/21/04, p. 5, lines 6-9). Furthermore, one of skill in the art would recognize homologs to be structurally similar genes and/or proteins that are part of a family whose members a conserved across a range of species. For example, the MAD family contains, but is not limited to, Yeast MAD2, *Xenopus* XMAD, Human hsMAD, and *Drosophila* MAD. Moreover, a standard definition of "homology" in the context of molecular biology refers to "[s]imilarity in structure of ... a molecule, reflecting a common evolutionary origin. Specifically, such similarity in protein or nucleic acid sequence." Molecular Biology of the Cell,

3rd Edition, 1994, p. G-12 (attached as Exhibit C). Additionally, the iHOP search referred to in **1.1.** *supra*, readily identifies MAD2 homologs (see link "Homologues of MAD2"; Ex. B) in humans (Exhibit B, Paper No. 1) , mouse (Exhibit B, Paper No. 2), Drosophila (Exhibit B, Paper No. 3), *C.elegans* (Exhibit B, Paper No. 4) and *Arabidopsis* (Exhibit B, Paper No. 5).

Moreover, Figure 1A of Li *et al.*, Science 1996, 274, 5285; 246-248 (of record) shows an amino acid sequence alignment of human, *Xenopus* and yeast MAD2 proteins. The alignment is further solid evidence that a person of ordinary skill in the art would readily understand the structural features of the MAD family of genes and know what constitutes a MAD2 homolog and what does not.

Additionally, "mutated MAD2 gene" refers to a variety of known and unknown mutant MAD2 alleles as well as mutants of MAD2 homologs in other species. The skilled artisan would have known that a mutated MAD2 gene could be the result of nucleic acid deletions, insertions or substitutions upstream, downstream, within or in between the MAD2 genomic coding regions. As such, it would not only be highly impractical to describe MAD2 mutants by way of a specific sequence as suggested by the Examiner; it would also be unnecessary because one of skill in the art would know to identify mutated MAD2 homologs because of the well described nature of the gene itself across a range of species.

Applicants have amended the claims to remove the term "analogs" of MAD2. Applicants respectfully assert that the amendments to claim 1 and the remarks above fully addresses this ground for rejection and withdrawal thereof is respectfully requested.

2. Rejection under 35 U.S.C. § 102

The Examiner rejects claims 1, 3-4 and 6-7 under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 5,972,640 to Drubin *et al.* (hereinafter 'Drubin') The Examiner argues that Drubin teaches a method for identifying an anti-mitotic compound comprising the steps of contacting a cell with an agent, wherein the cell has a functionally disrupted mitotic check point, *e.g.,* having at least one mutation in a MAD2 or TUB1 gene; and detecting the sensitivity or the response of the cells to the agent.

Furthermore, the Examiner states that the anti-mitotic activity of any identified compound is then confirmed using anti-tubulin immunofluoresence to identify spindle defects. Applicants respectfully disagree.

However, in order to expedite prosecution, Applicants have amended claim 1 to recite a method of identifying a drug that inhibits growth or replication of a cell having a mutated MAD2 gene or homolog thereof, the method comprising the steps of identifying a secondary gene comprising providing a plurality of cells having a genome, which includes at least one mutated MAD2 gene or homolog thereof; effecting one or more mutations in the genome of the cells, at one or more secondary genes; selecting those cells having at least one additional mutation that proves lethal to the cells only when the mutated MAD2 gene is present; determining a site in the genome of the cells in which the at least one lethal mutation is located, to provide a secondary gene; contacting a cell having a mutated MAD2 gene or homolog thereof with a drug; and identifying the drug by determining whether the drug modulates the activity of the wildtype secondary gene such that the drug is lethal to the cell having a mutated MAD2 gene but not to a wild type cell.

As such, claim 1 has now been amended to include a step for first identifying a secondary gene which is synthetically lethal when mutated and present in combination with a mutated MAD2 gene before the steps of screening candidate drug compounds. Drubin merely teaches a screen for antimitotic compounds that will selectively inhibit the growth of cells that are mutant in either a *mad* or *bub* gene. Applicants respectfully assert that Drubin does not teach identifying the claimed secondary genes. Additionally, Drubin does not teach identifying specific drugs by determining whether the drug modulates the activity of wildtype secondary genes such that the drugs are lethal to cells having a mutated MAD2 gene but not to a wild type cell.

Applicants respectfully submit that the foregoing amendments have fully addressed the Examiner's rejection and, therefore, request its removal.

3. Rejection under 35 U.S.C. § 103

The Examiner also rejects claims 1-7 under 35 U.S.C. § 103(a) as allegedly being unpatentably obvious over Drubin in view of Li *et al.*, Science 1996, 274, 5285; 246-248 (hereinafter 'Li'). The Examiner concedes that Drubin does not teach that the cells upon which the claimed methods may be performed can be tumor cells. However, Li allegedly teaches this connection.

Applicants respectfully submit that Drubin does not anticipate claims 1-7 for the reasons above because it does not teach the step of identifying secondary genes that are synthetically lethal

to MAD2 and selecting compounds by their ability to modulate wild type secondary genes. For the same reasons, Drubin does not render the claimed invention unpatentably obvious. For example, nothing Drubin teaches or suggests the identification of wild type genes which in mutant form are synthetically lethal in combination with mutant MAD2, let alone screening compounds that modulate such genes' wild type products. Moreover, Li contains no disclosure that overcomes the deficiencies of Drubin.

Applicants respectfully submit that the foregoing amendments and remarks have fully addressed the Examiner's rejections under 35 U.S.C. § 103 and, therefore, request their removal.

CONCLUSION

To the extent necessary, a petition for an extension of time under 37 C.F.R. § 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

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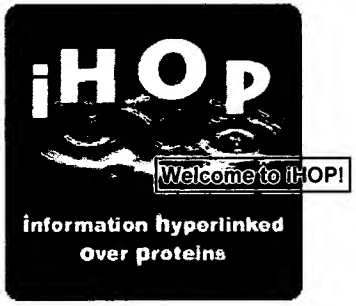

Symbol	Name	Synonyms	Organism
MAD2L1	Mitotic spindle assembly checkpoint protein MAD2A	HsMAD2, HSMAD2, MAD2, MAD2-like 1, MAD2 mitotic arrest deficient-like 1 (yeast)	Homo sapiens

UniProt	Q13257
IntAct	Q13257
PDB Structure	1DUJ, 1KLQ
OMIM	601467
NCBI Gene	4085
NCBI RefSeq	NP_002349
NCBI Accession	AAC50781, AAC52060, AAH00356

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However, Bub1 has several **functions** that are not shared with Mad2.

Mad2 phosphorylation **regulates** its association with Mad1 and the APC/C.

In addition, in vitro cdk1-phosphorylated Cdc20 **interacts** with Mad2 rather than APC/ C.

When expressed in wing imaginal disks, hSmad2 **induced** oversize wings while hSmad3 induced cell death.

In this model, the unattached **kinetochore** is postulated to catalytically convert Mad2 to a form that **binds** Cdc20.

Mutagenesis and NMR titration experiments show that a C-terminal flexible region of Mad2 is required for **binding** to Cdc20.

Upon checkpoint activation, Mad2 **binds** directly to Cdc20 and inhibits the anaphase-promoting complex or cyclosome (APC/C).

Using deletion mutagenesis and peptide mapping, we have identified the sequences in Cdc20 that **target** it to Mad2 and the APC, respectively.

In the course of these studies, a truncation mutant of Cdc20 (1-153) that constitutively **binds** Mad2 but fails to bind the APC was identified.

These data suggest that the phosphorylation state of Mad2 **regulates** its checkpoint activity by modulating its association with Mad1 and the APC/C.

The spindle checkpoint protein Mad1 **recruits** Mad2 to unattached kinetochores and is essential for Mad2-

Cdc20 complex formation in vivo but not in vitro.

We show that RNAi-mediated suppression of Mad1 **function** in mammalian cells causes loss of Mad2 kinetochore localization and impairment of the spindle checkpoint.

When the spindle-assembly checkpoint is activated, MAD2 forms a ternary **complex** with CDC20 and APC to prevent activation of APC, and thereby arrests cells at prometaphase.

A Mad2 mutant containing **serine** to **aspartic acid** mutations mimicking the C-terminal phosphorylation events fails to **interact** with **Mad1** or the APC/C and acts as a dominant-negative antagonist of wild-type Mad2.

We find HsMad1 **associated** with HsMad2.

Mad2 **binding** to Mad1 and Cdc20, rather than oligomerization, is required for the spindle checkpoint.

Mad2 also undergoes a similar striking structural change upon **binding** to a Mad1 or Cdc20 binding motif peptide.

A MHC-encoded ubiquitin-like protein (FAT10) **binds** noncovalently to the spindle assembly checkpoint protein MAD2.

The Mad2 spindle checkpoint protein undergoes similar major conformational changes upon **binding** to either Mad1 or Cdc20.

In this study, Mad2 and its **target** Slp1 were visualized in a tractable organism, fission yeast Schizosaccharomyces pombe.

top

We also show that Mad2 **associates** with the APC regulatory protein **p55Cdc** in mammalian cells as has been reported in yeast.

I show herein that in checkpoint-arrested cells, human **Cdc20** forms two separate, inactive complexes, a lower affinity complex with Mad2 and a higher affinity **complex** with **BubR1**.

The Sequestration Model postulates that Mad2 and BubR1 **bind** and sequester Cdc20, an APC/C activator, away from APC/C so substrates whose destruction drives mitotic exit are no longer ubiquitinated.

Our observations support a model in which Nuf2 and **Hec1 function** to prevent microtubule-dependent stripping of **Mad1** and Mad2 from **kinetochores** that have not yet formed stable kinetochore-microtubule attachments.

Reducing the levels of the checkpoint proteins BubR1 or Mad2 in human cancer cells or **inhibiting** BubR1 kinase activity provokes apoptotic cell death within six divisions except when cytokinesis is also inhibited.

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Symbol	Name	Synonyms	Organism
Mad2l1	Mitotic spindle assembly checkpoint protein MAD2A	MAD2, Mad2a, MAD2-like 1	Mus musculus

UniProt Q9JI53, Q9Z1B5
NCBI Gene 56150
NCBI RefSeq NP_062372
NCBI Accession AAD09238, AAF69525, AF259902

Homologues of Mad2l1 ... new

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Generation of Rb pathway lesions in normal and transformed cells **produces** aberrant Mad2 expression and mitotic defects leading to **aneuploidy**, such that elevated Mad2 contributes directly to these defects.

Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2.

The mouse homologue of Mad2 protein, which plays an important role in the M phase of the cell cycle, was revealed to associate with the box1/2 region specifically.

Mad2 protein was increased as a consequence of the absence of p53 function.

Mad2 is known to change affinity for binding partners cell cycle dependently.

Overexpression of Mad2 in meiosis I leads to a cell cycle arrest in metaphase I.

The increase in Mad2 protein was observed also at the cellular level by immunohistochemistry.

The Mad2 protein plays a significant role in accurate chromosome segregation in mitotic cells.

MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells.

In this report, we demonstrate that TCDD suppresses the expression of the checkpoint protein, Mad2.

MAD2 inhibits the anaphase-promoting complex when chromosomes are unattached to the mitotic spindle.

Dioxin suppresses the checkpoint protein, MAD2, by an aryl hydrocarbon receptor-independent pathway.

Deletion analysis indicated that interaction with box1/2 occurred through the C-terminal portion of Mad2.

Our data suggest that TCDD may increase chromosomal instability through the suppression of Mad2 expression.

Complete MAD2 inactivation has not been identified in human tumors, although partial defects are prevalent.

Differences in spindle association of the mitotic checkpoint protein **Mad2** in mammalian spermatogenesis and oogenesis.

The best producer among the several clones obtained was expanded and the secreted MAb (**MAD2**) purified and characterized.

Complete loss of the tumor suppressor **MAD2** causes premature **cyclin B** degradation and mitotic failure in human somatic cells.

Concordantly, **Mad2** is overexpressed in several tumour types, where it correlates with high E2F activity and poor patient prognosis.

In contrast to the situation in spermatogenesis, **Mad2** persisted at the kinetochores of normal, second-division oocytes at metaphase.

top

Furthermore, the mitotic checkpoint protein **Mad2** is present throughout meiotic maturation and is recruited to unattached kinetochores.

In addition, the association of **Mad2** with second-division-metaphase kinetochores differed significantly in male versus female meiosis.

Furthermore, **Mad2**+/- mice develop lung tumours at high rates after long latencies, implicating defects in the mitotic checkpoint in tumorigenesis.

This result indicates that loss of kinetochore-associated **Mad2** is not essential for the metaphase-to-anaphase transition during the first meiotic division.

Disruption of the male meiotic spindles with the microtubule depolymerizing agent nocodazole resulted in the appearance of **Mad2** at nearly all kinetochores.

In spermatogenesis, **Mad2** remained at most kinetochores throughout the entire first meiotic division and was lost only at metaphase of the second meiotic division.

We have investigated expression and subcellular localization of the spindle checkpoint protein **Mad2** during rat and mouse spermatogenesis and in superovulated mouse oocytes.

In contrast, the microtubule stabilizer taxol induced the loss of **Mad2** from the majority of the first-division-metaphase kinetochores in which it was normally present in untreated cells.

MAD2 and the panel of MAbs which are at present being purified may become a tool for studying the relevance of different domains of the anthracyclin molecule in terms of biologic activity.

Our immunofluorescence studies demonstrate substantial differences in the localization patterns of kinetochore-associated **Mad2** in these meiotic systems compared with previous studies of mitosis.

Thus, anaphase-promoting complex substrates exhibit distinct sensitivities in the presence of different **MAD2** doses, which in turn determine MAD2's role as either a tumor suppressor or an essential gene.

MAD2 cross-reacts to varying degrees with anthracycline compounds such as some DXR analogues and derivatives, but does not recognize anthracene and anthraquinone structures, with the exception of weakly reacting Mitoxantrone.

Expression of a dominant-negative **Mad2** protein interferes with proper spindle checkpoint

arrest.CONCLUSIONS: Errors in meiosis I cause missegregation of chromosomes and can result in the generation of aneuploid embryos with severe birth defects.

Second, the absolute rate of chromosome missegregation may be increased by alterations in the levels of two proteins, separase and **Mad2**, which are important for maintaining chromosomal segregation and the normal spindle checkpoint during mitosis.

By employing RNA interference in human somatic cells, we found that severe reduction of **MAD2** protein levels results in mitotic failure and extensive cell death arising from defective spindle formation, incomplete chromosome condensation, and premature mitotic exit leading to multinucleation.

In contrast, the postmitotic trophoblast giant cells survive without **Mad2**.

Cell cycle-dependent interaction of **Mad2** with conserved Box1/2 region of human granulocyte-macrophage **colony-stimulating factor** receptor common betac.

Double labelling **immunofluorescence** with **MAD-2** and a mouse monoclonal antibody against **vimentin**, the intermediate filament protein of cells of mesenchymal origin, showed coincidental staining which was distinct from that seen with antibodies against other cytoskeletal proteins.

At metaphase I and II, MC kinetochores assembled **MAD2** and **BUBR1** spindle checkpoint proteins.

These results show that the major tissue and leucocyte protein recognized by **MAD-2** is **vimentin**.

top

Using **immunoblotting**, both **MAD-2** and anti-vimentin reacted with a 55 kDa tissue component, and with purified **vimentin**.

Indeed, in survivin-depleted cells, **BubR1** and **Mad2** are prematurely displaced from kinetochores, yet no tension is generated at kinetochores.

We show that the mitotic checkpoint protein **Mad2** is a direct E2F target and, as a consequence, is aberrantly expressed in cells with **Rb** pathway defects.

Here we report that deletion of one **MAD2** allele results in a defective mitotic checkpoint in both human cancer cells and murine primary **embryonic** fibroblasts.

Cells overexpressing **Aurora-A** inappropriately enter anaphase despite defective spindle formation, and the persistence of **Mad2** at the kinetochores, marking continued activation of the spindle assembly checkpoint.

Northern analysis of two different spindle assembly checkpoint protein gene products from human, **BUB1** and **MAD2**, reveals an expression pattern with common tissue distribution consistent with roles in a common pathway.

First, Western blot analysis demonstrated that the levels of two proteins involved in regulating sister chromatid separation and the spindle checkpoint, **Mad2** and separase (**ESPL1**) were increased in null compared with WT cells.

The results of northern blot analysis for small **proline-rich protein 2** (Sprr2), 17beta-hydroxysteroid dehydrogenase type 2 (17betaHSD-2), high mobility group 2 (**Hmg2**), mitotic checkpoint component 2 (**Mad2**) and an EST AW555366 mRNA were consistent with that of microarray analysis.

Peptides corresponding to the box1 sequence also bound to **Mad2**, and mutation of the box1 decreased the **Mad2** interaction.

Thus, mRev3 could be required to repair a form of externally induced DNA damage that otherwise accumulates during clonal expansion or, consistent with the high homology shared between its **Rev7** partner and the mitotic checkpoint gene product **Mad2** [6], mRev3 might play a role in cell proliferation and genomic stability even in the absence of environmentally induced damage.

To determine the function of the mitotic checkpoint protein **Mad2** during normal cell division and when mitosis goes awry, we have knocked out **Mad2** in mice.

Suppression of **Mad2** was also observed in aryl hydrocarbon receptor-deficient mouse **embryonic** fibroblasts, suggesting that **TCDD** suppresses **Mad2** by a novel **TCDD receptor** signaling mechanism.

Binding affinity of **Mad2** to box1/2 increased in the late M phase, suggesting the possibility that **GM-CSF** participates in regulation of the M phase check point through interaction with **Mad2**.

Cenpag/g embryos assemble functional kinetochores that bind to a host of centromere-specific structural and mitotic spindle checkpoint proteins (**Cenpc**, **BubR1**, **Mad2** and **Zw10**).

We find that E5.5 **embryonic** cells lacking **Mad2**, like **mad2** yeast, grow normally but are unable to arrest in response to spindle disruption.

Cyclin B is degraded prematurely in the **MAD2** short interfering RNA-treated cells but not in **MAD2**+/- cells, suggesting an explanation for the spindle failure and mitotic catastrophe in the **MAD2** knockdown cells.

Antisera to **CENP-A**, **CENP-B**, **CENP-C**, **CENP-E**, CENP-F, **INCENP**, **CLIP-170**, dynein, dynactin subunits **p150** (**Glued**) and **Arp1**, **MCAK**, **Tsg24**, **p55CDC**, **HZW10**, **HBUB1**, **HBUBR1**, **BUB3**, **MAD2**, **ERK1**, 3F3/2, topoisomerase II and a murine **HP1** homologue, **M31**, were used in immuno-fluorescence experiments in conjunction with FISH employing specific DNA probes to clearly identify neocentromeric DNA.

Symbol	Name	Synonyms	Organism
CG17498	CG17498-PA		Drosophila melanogaster

NCBI Gene 38656
NCBI Accession AAF50740, AAL48090, AE003565

Homologues of CG17498 ... new

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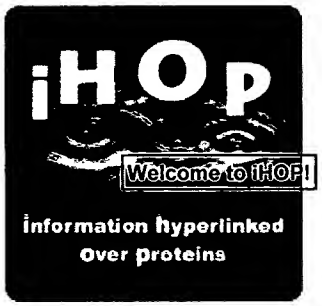
The annotation process is automatic and misidentification of gene symbols cannot be ruled out completely. The main reason for missing a gene is that a synonym was not given in the corresponding database (e.g. HUGO, LocusLink, etc.) and could therefore not be considered in the annotation process. Moreover, synonym ambiguity makes the correct automatic identification sometimes impossible.

Experimental evidence for the association between **CG17498** and **CG32000** from **Y2H**.

Experimental evidence for the association between **CG17498** and **CG15576** from **Y2H**.

Experimental evidence for the association between **CG17498** and **CG13214** from **Y2H**.



Symbol	Name	Synonyms	Organism
mdf-2		CELK04085, Y69A2AR.30a, Y69A2AR.30b	Caenorhabditis elegans

NCBI Gene 177046
NCBI Accession AAF63495, AAK68576, AAM15619

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Antibodies that recognize the **MDF-2** protein localize to nuclei of the **cleaving** embryo in a cell-cycle-dependent manner. **mdf-1**, a gene encoding a product that interacts with **MDF-2**, is required for cell-cycle arrest and proper chromosome segregation in premeiotic **germ cells** treated with **nocodazole**, a microtubule-depolymerizing agent.

Here we show that **mdf-1** and **mdf-2** are components of the spindle-assembly checkpoint in Caenorhabditis elegans, and are essential for the long-term survival and fertility of this organism.

During anoxia-induced suspended animation, embryos lacking functional SAN-1 or a second spindle checkpoint component, **MDF-2**, failed to arrest the cell cycle, exhibited chromosome missegregation, and showed reduced viability.

Experimental evidence for the association between **mdf-2** and **1G243** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **2M633** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **1C653** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **5D165** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **1I177** from **Y2H**.

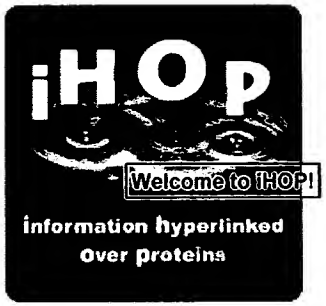
Experimental evidence for the association between **mdf-2** and **tcl-2** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **csn-5** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **3K840** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **5M843** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **1L214** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **5O926** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **air-1** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **3J401** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **2I64** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **cpr-4** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **hum-5** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **2L733** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **1C869** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **apt-2C** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **tbp-1** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **3E52** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **5N852** from **Y2H**.
Experimental evidence for the association between **mdf-2** and **4E167** from **Y2H**.



Symbol	Name	Synonyms	Organism
At3g25980		MPE11.16	Arabidopsis thaliana
NCBI Gene	822195		

Homologues of **At3g25980** ... **new**

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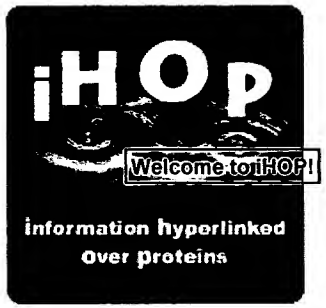

Symbol	Name	Synonyms	Organism
TUB1	Tubulin alpha-1 chain	YML085C	Saccharomyces cerevisiae

UniProt P09733
NCBI Gene 854889
NCBI RefSeq NP_013625.1
NCBI Accession AAA35180, CAA86653

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More literature information for this gene ...

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Filaments of cyano fluorescent protein-tagged **Fin1** **colocalize** with filaments of green fluorescent protein-tagged **Tub1** only in large-budded cells.

A systematic study of 51 **tub1** alleles suggests a correlation between specific failure to **interact** with Bim1p in the **two-hybrid** **assay** and synthetic lethality with the bim1Delta allele.

A previously uncharacterized yeast gene (**YER016w**) that we have named **BIM1** (**binding** to **microtubules**) was obtained from a two-hybrid screen of a yeast cDNA library using as bait the entire coding sequence of **TUB1** (encoding **alpha-tubulin**).

Aut7p is **attached** to microtubules via Aut2p, which interacts with tubulins **Tub1**p and Tub2p. **aut2**- and aut7-deleted cells are unable to deliver autophagic vesicles and the precursor of aminopeptidase I to the **vacuole**.

This arrest is due to lower levels of **alpha-tubulin**, a protein encoded by **TUB1** and **TUB3**, two intron-containing genes.

However, the **mcm19** null mutation conferred growth defects in the presence of a mutation in the **TUB1** gene coding for alpha-tubulin.

Tubulin was purified from wild-type and deletion strains lacking either **Tub1** or **Tub3**, and parameters of **microtubule** dynamics were examined.

This technique was used to tag Cdc3 and **Tub1** with GFP, YFP and CFP, which were readily visualized by fluorescence microscopy and localized as expected.

Mutations that suppressed the cold-sensitive phenotypes of two of the **TUB1** alleles occurred in TUB2, the single structural gene specifying **beta-tubulin**.

Recent investigations have confirmed the presence of one **alpha-tubulin** gene (**TUB1**) and one **beta-tubulin** gene (TUB2) in the dimorphic fungus Histoplasma capsulatum.

The noncomplementation between **tub1** and tub2 mutations is gene specific and allele specific, suggesting that the phenotype is due to an interaction at the protein level.

The yeast Saccharomyces cerevisiae has two **alpha-tubulin** genes, **TUB1** and TUB3, either of which alone is sufficient for these processes when present in a high enough copy number.

Genomic replacement of an intronless **TUB1** gene relieves the benomyl sensitivity of prp17 mutants; however, they remain temperature sensitive, implying multiple limiting factors for mitosis.

Nucleotide sequencing studies revealed that the two genes, named **TUB1** and TUB3, encoded gene products of 447 and 445 amino acids, respectively, that are highly homologous to alpha-tubulins from other species.

The systematic **tub1** mutations were placed, along with the comparable set of **tub2** mutations previously described, onto a model of the yeast alpha-beta-tubulin dimer based on the three-dimensional structure of bovine **tubulin**.

The relative importance in these processes of the two divergent **alpha-tubulin** genes of the budding yeast Saccharomyces cerevisiae, **TUB1** and TUB3, was examined through the construction of null mutations and by increasing their copy number on chromosomes and on plasmids.

The yeast Saccharomyces cerevisiae has two genes for alpha-tubulin, **TUB1** and TUB3, and one beta-tubulin gene, TUB2.

Splicing of **TUB1** and TUB3 transcripts, which encode **alpha-tubulin**, was analyzed in prp17 and other second-step factor mutants.

Transformation of mutant cells with genomic libraries repeatedly identified three different suppressors: the two wild-type **alpha-tubulin** genes, **TUB1** and TUB3; and BUB3.

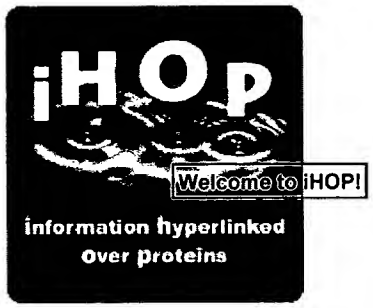
Genetic interactions between stu1 alleles and alleles of **TUB1** and TUB2 suggest that Stu1p specifically interacts with **microtubules**.

top

In order to ascertain associations of other tubulins with dinitroaniline resistance, four **beta-tubulin** cDNA classes (designated **TUB1**, TUB2, TUB3, and TUB4) were isolated from dinitroaniline-susceptible and -resistant biotypes.

TUB1 splicing is inefficient in prp17, prp16 and prp22, and marginally affected in prp18, slu7-1 and psf1-1.

Such mutants fall into six complementation groups: **TUB1**, TUB2 and TUB3, the three **tubulin** genes of yeast, and three new genes, which we have named **CIN1**, CIN2 and CIN4.



Symbol	Name	Synonyms	Organism
MAD2	Mitotic spindle checkpoint component MAD2	J1256, Mitotic MAD2 protein, YJL030W	Saccharomyces cerevisiae

UniProt	P40958
NCBI Gene	853422
NCBI RefSeq	NP_012504.1
NCBI Accession	AAA21385, CAA89321

Homologues of MAD2 ... new

More literature information for this gene ...

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Comparisons of the amino acid sequences suggest that the proteins encoded by the Saccharomyces cerevisiae genes **MAD2** and **BET2** are the yeast counterparts of the mammalian Rab GG **transferase** alpha and beta subunits, respectively.

Overexpression of mph1, like overexpression of **mad2**, mimics activation of the checkpoint and imposes a metaphase arrest. mph1 protein shares sequence similarity with **Mps1**p, a dual specificity kinase that functions in the spindle checkpoint of the budding yeast Saccharomyces cerevisiae.

Bipolar orientation of chromosomes in Saccharomyces cerevisiae is monitored by **Mad1** and **Mad2**, but not by **Mad3**.

In contrast, we show that whereas Saccharomyces cerevisiae **Bub1**p and **Bub3**p are bound to kinetochores early in mitosis as part of the normal cell cycle, **Mad1**p and **Mad2**p are kinetochore bound only in the presence of spindle damage or kinetochore lesions that interfere with chromosome-microtubule attachment.

Bet2p forms a **complex** with **Mad2**p that appears to bind geranylgeranyl pyrophosphate, but not farnesyl pyrophosphate.

Budding yeast **Bub2** is localized at spindle pole bodies and **activates** the mitotic checkpoint via a different pathway from **Mad2**.

A MHC-encoded ubiquitin-like protein (**FAT10**) **binds** noncovalently to the spindle assembly checkpoint protein **MAD2**.

We also show that **Mad2** **associates** with the APC regulatory protein **p55Cdc** in mammalian cells as has been reported in yeast.

Yeast two-hybrid screening of a human lymphocyte library and immunoprecipitation studies revealed that **FAT10** noncovalently **associated** with **MAD2**, a protein implicated in a cell-cycle checkpoint for spindle

assembly during anaphase.

Genetic interactions with checkpoint and apc mutants suggest Cdc28 may **regulate** checkpoint arrest downstream of the **MAD2** and **BUB2** pathways.

On the basis of these results and genetic analysis of double mutants, we propose a model in which **Mad1**p bound to a Nup53p-containing **complex** sequesters **Mad2**p at the NPC until its release by activation of the spindle checkpoint.

The **Cdc14** liberation from the nucleolus was **inhibited** by the **Mad2** checkpoint and by the **Bub2** checkpoint in a different manner when microtubule organization was disrupted.

Mad1p forms a tight **complex** with **Mad2**p [7], and has been shown to recruit **Mad2**p to kinetochores [8].

Analysis of the genetic interaction of **BFA1** with known mitotic checkpoint genes suggest that **Bfa1** **functions** in the same pathway with **Bub2** but not with **Mad1** or **Mad2**.

In addition, we find that **Mad3**p **interacts** with **Mad2**p and the cell cycle regulator **Cdc20**p.

MAD3 encodes a novel component of the spindle checkpoint which **interacts** with **Bub3**p, **Cdc20**p, and **Mad2**p.

The following observations indicate that **Bub2** and **Mad1**, 2 probably activate the checkpoint via different pathways: (a) unlike the other Mad and Bub proteins, **Bub2** localizes at the spindle pole body (SPB) throughout the cell cycle; (b) the **effect** of concomitant lack of **Mad1** or **Mad2** and **Bub2** is additive, since nocodazole-treated mad1 bub2 and mad2 bub2 double mutants rereplicate DNA more rapidly and efficiently than either single mutant; (c) cell cycle progression of **bub2** cells in the presence of nocodazole requires the **Cdc26** APC subunit, which, conversely, is not required for **mad2** cells in the same conditions.

In addition, **Mad2**p **binds** to all of the different **phosphorylated** isoforms of **Mad1**p that can be resolved on SDS-PAGE.

Checkpoint proteins **Mad2**p and Mad3p/BubR1p **bind** and **inhibit** **Cdc20**p, an activator for the anaphase-promoting complex (APC).

In extracts prepared from M phase, but not from interphase **HeLa cells**, **p55CDC** **coimmunoprecipitates** with three important elements of the M phase checkpoint machinery: **Cdc27**, **Cdc16**, and **Mad2**. **p55CDC** is required for **binding** **Mad2** with the **Cdc27** and **Cdc16**.

top

The spindle checkpoint of budding yeast depends on a tight complex between the **Mad1** and **Mad2** proteins.

Therefore **MAD2** and **BET2** gene products may physically interact to form a geranylgeranyl **transferase** complex.

Studies in yeast have shown that binding of **MAD1** to **MAD2** is important for the checkpoint function of the latter.

RAM2 is also homologous to **MAD2**, a yeast gene whose product has been implicated in the feedback control of mitosis.

MAD2beta has 23% sequence identity with **MAD2**, which is a component of the spindle assembly (or mitotic) checkpoint mechanism.

In the same screen which led to the isolation of **mad2** and mph1, we also isolated dph1, a **cDNA** that encodes a protein 46% identical to an S. cerevisiae SPB duplication protein, **Dsk2**p.

In this context, the mitotic arrest deficiency protein 2 (**MAD2**) censors chromosomal mis-segregation by monitoring microtubule attachment/tension, a role that requires its attachment to kinetochores.

We have identified mitotic arrest deficient 2 (**MAD2**) as a binding partner of the TACE cytoplasmic domain, and a novel MAD2-related protein, MAD2beta, as a binding partner of the MDC9 cytoplasmic domain.

This arrest depends on the spindle checkpoint protein **Mad2**.

The 572 del A mutation creates a truncated **MAD2** protein product.

Mad2 transiently associates with an APC/p55Cdc complex during mitosis.

The mitotic arrest defects of ibd2Delta were not recovered by **MAD2**, or vice versa.

We cloned **MAD2**, which encodes a putative calcium-binding protein whose disruption is lethal.

The **kinetochore** is an integral component of the **MAD2** branch of the spindle checkpoint pathway.

Microinjection of antibody to **Mad2** protein into mammalian cells in mitosis induces premature anaphase.

We support the idea that **MAD2** is attachment-sensitive and that tension stabilizes **microtubule** attachments.

Most current models of spindle assembly checkpoint signaling involve inhibition of the Cdc20-APC by **Mad2** protein.

We also found that the delay in mitotic exit in mutants with misoriented spindles depended on **BUB2** and **BFA1**, but not on **MAD2**.

We have cloned the **MAD2** gene, which encodes a protein of 196 amino acids that remains at a constant level during the cell cycle.

These studies suggest that **Mad2** protein function is essential for the timing of anaphase onset in somatic cells at each mitosis.

top

Further, the tension-sensitive 3F3/2 phosphoepitope colocalized, and was lost concomitantly, with **MAD2** staining at the meiotic **kinetochore**.

Interestingly, we found that in the yeast two-hybrid system, the interaction of F2 and **MAD2** was stronger than that of intact p55CDC/hCDC20.

We tested whether the **Mad2** protein participates in regulating the timing of anaphase onset in mammalian cells in the absence of microtubule drugs.

Two recent papers report the cloning of human and Xenopus homologues of one of these yeast genes, called **MAD2** (for mitotic arrest deficient-2)(1,2).

The maize homologue of the cell cycle checkpoint protein **MAD2** reveals **kinetochore** substructure and contrasting mitotic and meiotic localization patterns.

Interaction of **MAD2** with the carboxyl terminus of the **insulin receptor** but not with the IGFIR. Evidence for release from the **insulin receptor** after activation.

Introduction of antibodies to the **MAD2** protein into living mammalian cells or Xenopus egg extracts abrogates the M phase arrest induced by microtubule inhibitors.

In yeast, the **Mad2** protein is required for the M phase arrest induced by microtubule inhibitors, but the protein is not essential under normal culture conditions.

Finally, T47D, a human breast tumor cell line that is sensitive to **taxol** and **nocodazole**, had reduced **MAD2** expression and failed to arrest in mitosis after **nocodazole** treatment.

MAD2 staining was primarily observed on mitotic **kinetochores** that lacked attached **microtubules**; i.e., at prometaphase or when the **microtubules** were depolymerized with oryzalin.

Unattached **kinetochores** are believed to release an activated form of **Mad2** that inhibits APC/C-dependent ubiquitination and subsequent proteolysis of components needed for anaphase onset.

These results suggest a model for the regulation of the APC by **Mad2** and may explain how the spindle assembly checkpoint apparatus controls the timing of mitosis under normal growth conditions.

When this condition is not met, **MAD2**, a component of the spindle checkpoint complex, associates with p55CDC/hCDC20 to inhibit ubiquitination of substrates by the anaphase-promoting complex (APC).

Overexpression of **BUB1**, which interacts genetically with **BUB3** and which is involved in the same checkpoint pathway, also rescues the cold sensitivity of tub1-729, but another checkpoint gene, **MAD2**, does not.

In contrast, the loss of **MAD2** staining in meiosis was not correlated with initial **microtubule** attachment but was correlated with a measure of tension: the distance between homologous or sister **kinetochores** (in meiosis I and II, respectively).

Immunofluorescence studies reveal that cells blocked in metaphase by chromosome damage contain one or more Mad2-positive kinetochores, and the block is rapidly overridden when the cells are microinjected with a dominant-negative construct of **Mad2** (Mad2deltaC).

Thus, it is likely that **p55CDC** mediates the association of **Mad2** with the cyclosome/anaphase-promoting complex.

Bet2p and **Mad2p** are components of a prenyltransferase that adds geranylgeranyl onto **Ypt1p** and **Sec4p** [see comments].

We have also found that the **MAD2** but not the **BUB2** spindle checkpoint gene is required for efficient arrest of yku70Delta mutants.

One pathway, defined by **MAD2**, controls the metaphase-to-anaphase transition and the other, defined by **BUB2**, controls the exit from mitosis [3-6].

top

The **MAD2** branch of the pathway responds to kinetochore microtubule interactions and the **BUB2** branch

of the pathway operates within the cytoplasm, responding to spindle misorientation.

The second one, that requires FZY function (even in the absence of MAD2 protein and when the spindle assembly checkpoint is not activated) is not yet understood at its molecular level.

Mammalian p55CDC mediates association of the spindle checkpoint protein Mad2 with the cyclosome/anaphase-promoting complex, and is involved in regulating anaphase onset and late mitotic events.

The yeast MAD2 protein was first identified in a genetic screen to identify cell cycle checkpoint regulatory proteins, yet the mechanism by which MAD2 functions in cell cycle control is currently unclear.

Here we isolate a new temperature-sensitive mad2 mutant, mad2-2ts, and find that Mad2p is required for the membrane association of Ypt1p and Sec4p, two prenylated small GTP-binding proteins involved in protein trafficking.

Evidence for an interaction of the metalloprotease-disintegrin tumour necrosis factor alpha convertase (TACE) with mitotic arrest deficient 2 (MAD2), and of the metalloprotease-disintegrin MDC9 with a novel MAD2-related protein, MAD2beta.

The independent identification of MAD2 and MAD2beta as potential interacting partners of distinct metalloprotease-disintegrins raises the possibility of a link between metalloprotease-disintegrins and the cell cycle, or of functions for MAD2 and MAD2beta that are not related to cell cycle control.

In contrast, the postmitotic trophoblast giant cells survive without Mad2.

Blocking APCCdh1-mediated Clb2 proteolysis and chromosome re-duplication does not require Mad2 but a different protein, Bub2.

Gel filtration and co-immunoprecipitation analyses reveal that Mad2p tightly associates with another spindle checkpoint component, Mad1p.

The normal centromere has been deleted and a neocentromere containing CENP-A, -C, -E and Mad2 but not CENP-B has formed close to the breakpoint.

We show that two proteins required for the execution of the spindle checkpoint, Mad1p and Mad2p, reside predominantly at the NPC throughout the cell cycle.

We show that spindle damage blocks sister chromatid separation solely by inhibiting APCCdc20-dependent Pds1 proteolysis and that this process requires Mad2.

Moreover, although Mad1p and Mad2p perform essential mitotic functions during every division cycle in mammalian cells, they are required in budding yeast only when mitosis goes awry.

In the present report, we demonstrate that the inhibitory association between Mad2 and the APC component Cdc27 also takes place transiently during the early stages of a normal mitosis and is lost before mitotic exit.

Hierarchical clustering analysis of the set1(-) expression profile revealed a correspondence to that of a mad2(-) strain, suggesting that the transcriptional defect in the set1(-) strain may be due to changes in chromatin structure.

Here we show that in budding yeast, BUB2 and BFA1 are also required for the maintenance of G2/M

arrest in response to DNA damage and to spindle misorientation. cdc13-1 **bub2** and cdc13-1 **bfa1** but not cdc13-1 **mad2** double mutants rebud and reduplicate their DNA at the restrictive temperature.

We identified a human cDNA encoding a protein that appears to be the human homolog of the yeast **MAD2** protein, which we term **hMAD2**.

Since high levels of **Mad2** overcome checkpoint loss in Mps1-depleted extracts, **Mps1** acts upstream of Mad2-mediated inhibition of APC/C.

The checkpoint response, however, is significantly weakened in Sgt1-depleted cells, and this correlates with a dramatic reduction in kinetochore levels of **Mad1**, **Mad2** and **BubR1**.

top

Northern analysis of two different spindle assembly checkpoint protein gene products from human, **BUB1** and **MAD2**, reveals an expression pattern with common tissue distribution consistent with roles in a common pathway.

Human, or Homo sapiens, **MAD2** (**hsMAD2**) was localized at the **kinetochore** after chromosome condensation but was no longer observed at the **kinetochore** in metaphase, suggesting that **MAD2** might monitor the completeness of the spindle-kinetochore attachment.

We find that three genes (**MAD2**, **BUB1**, and **BUB2**) that are required for the spindle assembly checkpoint in budding yeast (defined by antimicrotubule drug-induced arrest or delay) are also required in the establishment and/or maintenance of kinetochore-induced delays.

Like **Bet2p** and **Mad2p**, **Mrs6p** is required for the membrane attachment of **Ypt1p** and **Sec4p** in vivo.

Checkpoint arrest in the absence of bipolar orientation and tension (induced by replication block in a **cdc6** mutant) was lacking in cells without **MAD1** or **MAD2**.

Deletion and mutational analysis of both proteins indicate that association of **Mad2p** with **Mad1p** is critical for checkpoint function and for hyperphosphorylation of **Mad1p**.

Whereas the **MAD2** and **BUB1** genes were absolutely required for delay, loss of **BUB2** function resulted in a partial delay defect, and we suggest that **BUB2** is required for delay maintenance.

Combined immunolocalization of **MAD2** and a recently cloned maize **CENPC** homologue indicates that **MAD2** localizes to an outer domain of the prometaphase **kinetochore**.

In budding yeast, **Mad1**, **Mad2**, and **Mad3** proteins are equally required for arrest in the presence of damage induced by antimicrotubule drugs or catastrophic loss of spindle structure.

Mps1 is essential for the checkpoint because it is required for recruitment and retention of active CENP-E at **kinetochores**, which in turn is necessary for **kinetochore** association of **Mad1** and **Mad2**.

On activation of the spindle checkpoint, we detect changes in the interactions between these proteins, including the release of **Mad2p** (but not **Mad1p**) from the NPC and the accumulation of **Mad2p** at **kinetochores**.

Thus, mRev3 could be required to repair a form of externally induced DNA damage that otherwise accumulates during clonal expansion or, consistent with the high homology shared between its **Rev7** partner and the mitotic checkpoint gene product **Mad2** [6], mRev3 might play a role in cell proliferation and

genomic stability even in the absence of environmentally induced damage.

Mad2 and **Mad3** coprecipitated with **Cdc20** at all stages of the cell cycle.

We conclude that **Mad1** and **Mad2** are required to detect bipolar orientation and/or tension at **kinetochores**, whereas **Mad3** is not.

Therefore, mitotic arrest induced by excess **Mps1p** expression is due to the action of the **MAD2** branch of the spindle checkpoint pathway and excess **Mps1p** acts downstream of the **kinetochore**.

We determined which spindle defects the checkpoint can detect by examining the interaction of mutations that compromise the checkpoint (**mad1**, **mad2**, and **mad3**) with those that damage various structural components of the spindle.

To determine the function of the mitotic checkpoint protein **Mad2** during normal cell division and when mitosis goes awry, we have knocked out **Mad2** in mice.

CDH1 showed genetic interactions with **MAD2** and **PDS1**, genes encoding components of the mitotic spindle assembly checkpoint that acts at metaphase to prevent premature chromosome segregation.

We show that low concentrations of **nocodazole** delay cell division under the control of the previously identified mitotic checkpoint genes **BUB1**, **BUB3**, **MAD1**, and **MAD2** and independently of **BUB2**.

Analysis of **Mad1p** phosphorylation in other spindle assembly checkpoint mutants reveals that this response to microtubule-disrupting agents is defective in some (**mad2**, **bub1**, and **bub3**) but not all (**mad3**, **bub2**) mutant strains.

top

We find that formation of this complex requires **Mad2p** and **Mps1p** but not **Mad3p** or **Bub2p**.

In the two-hybrid system, three proteins that are components of the checkpoint, **Mad1**, **Mad2**, and **Mad3**, were shown to interact with **Cdc20**, a protein required for exit from mitosis.

Our data imply that **Mad1**, **Mad2**, **Mad3** and **Bub1** regulate APCCdc20, whereas **Bub2** regulates APCCdh1.

We find that E5.5 **embryonic** cells lacking **Mad2**, like **mad2** yeast, grow normally but are unable to arrest in response to spindle disruption.

This process requires the association between **Cdc20p** and **Mad2p**, and functional APC, but is independent of the known destruction boxes in **Cdc20p** and the other APC activator **Cdh1p**.

We have examined the status of proteins associated with the checkpoint protein complex (**BUB1**, **BUBR1**, **BUB3**, **MAD2**), the anaphase-promoting complex (**Tsg24**, **p55CDC**), and other proteins associated with mitotic checkpoint control (**ERK1**, 3F3/2 epitope, **hZW10**), on a human dicentric **chromosome**.

The pattern of **kinetochore** localization of **hMPS1** in CENP-E defective cells suggests that their interaction with the **kinetochore** is sensitive to microtubule occupancy rather than **kinetochore** tension. **hMPS1** is required for **MAD1**, **MAD2** but not **hBUB1**, **hBUBR1** and **hROD** to bind to **kinetochores**.

The binding of **Mad2** depended on **Mad1** and that of **Mad3** on **Mad1** and **Mad2**.

Similar to cells overexpressing **MPS1**, the BUB1-5 delay was dependent upon the functions of the other

checkpoint genes, including **BUB2** and **BUB3** and **MAD1**, **MAD2**, and **MAD3**.

The cold sensitivity of tub1-729 is suppressed by extra copies of a subset of the mitotic checkpoint genes **BUB1**, **BUB3**, and **MPS1**, but not **MAD1**, **MAD2**, and **MAD3**.

Experimental evidence for the association between **MAD2** and **MGS1** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **EBS1** from **Y2H**.

Experimental evidence for the association between **MAD2** and **NUP157** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **SAP4** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **SIN4** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **GLO3** from **IntAct**.

Experimental evidence for the association between **MAD2** and **AHA1** from **Y2H**.

Experimental evidence for the association between **MAD2** and **MSB2** from **IntAct & Y2H**.

MOLECULAR BIOLOGY OF
THE CELL
THIRD EDITION

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

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homologous chromosome (homologue)
One of two copies of a particular chromosome in a diploid cell, each copy being derived from a different parent.

homology
Similarity in structure of an organ or a molecule, reflecting a common evolutionary origin. Specifically, such a similarity in protein or nucleic acid sequence. Contrasted with analogy—a similarity that does not reflect a common evolutionary origin.

homozygote
Diploid cell or organism having two identical alleles of a specified gene.

housekeeping gene
Gene serving a function required in all the cell types of an organism, regardless of their specialized role.

hybridization
Process whereby two complementary nucleic acid strands form a double helix during an annealing period; a powerful technique for detecting specific nucleotide sequences.

hybridoma
Cell line used in the production of monoclonal antibodies; obtained by fusing antibody-secreting B lymphocytes with cells of a lymphocyte tumor.

hydrocarbon
Compound that has only carbon and hydrogen atoms.

hydrolysis (adjective **hydrolytic**)
Cleavage of a covalent bond with accompanying addition of water, —H being added to one product of the cleavage and —OH to the other.

hydrophilic
Polar molecule or part of a molecule that forms enough hydrogen bonds to water to dissolve readily in water. (Literally, "water loving.")

hydrophobic (lipophilic)
Nonpolar molecule or part of a molecule that cannot form favorable bonding interactions with water molecules and therefore does not dissolve in water. (Literally, "water hating.")

hydroxyl (—OH)
Chemical group consisting of a hydrogen atom linked to an oxygen, as in an alcohol.

hypertonic
Describes any medium with a sufficiently high concentration of solutes to cause water to move out of a cell due to osmosis. (From Greek *huper,* over.)

hypotonic
Describes any medium with a sufficiently low concentration of solutes to cause water to move into a cell due to osmosis. (From Greek *hupo,* under.)

immortalization
Production of a cell line capable of an unlimited number of cell divisions. Can be the result of a chemical or viral transformation or of fusion with cells of a tumor line.

immune response
Response made by the immune system of a vertebrate when a foreign substance or microorganism enters its body.

immune system
Population of lymphocytes and other white blood cells in the vertebrate body that defends it against infection.

immunoglobulin (Ig)
An antibody molecule. Higher vertebrates have five classes of immunoglobulin—IgA, IgD, IgE, IgG, and IgM—each with a different role in the immune response.

immunoglobulin like (Ig-like) domain
Characteristic protein domain of about 100 amino acids that is found in antibody molecules and in many other proteins that form the Ig superfamily.

***in situ* hybridization**
Technique in which a single-stranded RNA or DNA probe is used to locate a gene or an mRNA molecule in a cell or tissue. (*See also* **hybridization**.)

in vitro
Term used by biochemists to describe a process taking place in an isolated cell-free extract. Also used by cell biologists to refer to cells growing in culture (*in vitro*), as opposed to in an organism (*in vivo*). (Latin for "in glass.")

in vivo
In an intact cell or organism. (Latin for "in life.")

induction (embryonic)
Change in the developmental fate of one tissue caused by an interaction with another tissue.

inflammatory response
Local response of a tissue to injury or infection. Caused by invasion of white blood cells, which release various local mediators such as histamine.

initiation factor
Protein that promotes the proper association of ribosomes with mRNA and is required for the initiation of protein synthesis.

inositol
Cyclic molecule with six hydroxyl groups that forms the hydrophilic head group of inositol phospholipids.

inositol phospholipids (phosphoinositides)
One of a family of lipids containing phosphorylated inositol derivatives. Although minor components of the plasma membrane, they are important in signal transduction in eucaryotic cells. (*See* Figure 15–29.)

insulin
Polypeptide hormone that is secreted by β cells in the pancreas and helps regulate glucose metabolism in animals.

integrin
Member of the large family of transmembrane proteins involved in the adhesion of cells to the extracellular matrix.

interleukin
Secreted peptide or protein that mainly mediates local interactions between white blood cells (leucocytes).

intermediate filament
Fibrous protein filament (about 10 nm in diameter) that forms ropelike networks in animal cells. One of the three most prominent types of cytoskeletal filaments.

internal membrane
Eucaryotic cell membrane other than the plasma membrane. The membranes of the endoplasmic reticulum and the Golgi apparatus are examples.